Swedish University of Agricultural Sciences
The Faculty of Natural Resources and Agricultural Sciences
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Kwabena Owusu Baffoe

30 ECTS hec • Advanced D
Master of Science in Forests as a Natural Resources • Master's thesis, Department of Ecology: 2011:12
Uppsala 2011

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Kwabena Owusu Baffoe

Supervisor: Johan A Stenberg, Swedish University of Agricultural Sciences, Department of Ecology
Email: johan.stenberg@slu.se

Assistant Supervisor: Peter Dalin, Swedish University of Agricultural Sciences, Department of Ecology
Email: peter.dalin@slu.se

Examiner: Göran Nordlander, Swedish University of Agricultural Sciences, Department of Ecology
Email: goran.nordlander@slu.se

Credits: 30 ECTS hec
Level: Advanced D
Course title: Independent project / Degree project in Biology D
Course code: EX0564
Programme/education: Master of Science in Forests as a Natural Resources

Place of publication: Uppsala
Year of publication: 2011
Title of series: no: Master's thesis, Department of Ecology: 2011:12
Online publication: http://stud.epsilon.slu.se

Key Words: temperature; development rate; body size; development time; Braconidae; *Perilitus brevicolis; Phratora vulgatissima*; host-parasitoid interactions; bodyguard, biological control; *Salix*

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Abstract

The influence of temperature on the performance of the willow bodyguard, *Perilitus brevicollis* Haliday, 1835 (Hymenoptera: Braconidae) as well as the impact of temperature and parasitism on its host feeding capacity were assessed. The study also aimed at identifying the similarities and differences among two populations (Swedish and Irish) with regards to parasitoid diversity, size and parasitism rate. To achieve the former objective of the study, *P. brevicollis* parasitized adult *Phratora vulgatissima* were reared under four constant temperatures (10, 15, 20, 25 ℃) in the laboratory. It was then revealed that: (i) development rate was faster at the higher temperatures and vice versa; (ii) larger adult parasitoid size were achieved at the lower temperature (10 ℃) and smaller at the highest temperatures (25 ℃); (iii) 20 ℃ was found to be an optimal temperature for survival; (iv) host feeding capacity was reduced by the combine effect of parasitism and temperature especially at the higher temperatures. To achieve the second objective, overwintering adult beetles were collected from two sites in Sweden and Ireland and were reared in the greenhouse. The result indicated that, *P. brevicollis* was common to the two countries with no other species identified. These results from the two experiments underlie the importance of temperature in rearing the parasitoid for biological control program as well as predicting the performance of the parasitoid in future climates. The result also serves as basis for choosing parasitoid to control *Phratora* beetles in different geographical areas.

Introduction

Global climate warming has become eminent as global temperature is reported to have increased by 0.6°C during the past century and the climate is predicted to continue warming between the ranges of 1.0-3.5°C with an average of 2°C until the year 2100 (Bruce *et al*., 1996). Mean surface temperature of the earth is expected to increase if emissions continue to accelerate (Bale *et al*., 2002). The importance of climate change with respect to its impact on the higher trophic levels that depend on the ability of the lower trophic levels in adapting to climate change situations cannot be overlooked. Understanding the effects of climate variability on the third trophic level is crucial in forecasting or in using parasitoids in biological controls (Harrington *et al.,* 2001; Landsberg and Smith, 1992). Increased average temperature is not the only anticipated result from global climate change but also increase in intensity and frequency of extreme climatic events like heat waves (Esterling *et al,* 2000).

Exposure to temperatures below the minimal required temperature causes development among insects to cease (Hance *et al*., 2007). Larval instars are either increased or decreased by low temperatures. Study conducted by Denlinger & Lee (1998) revealed that *Ephestia kuehniella* had four instars instead of five when reared at 18 ℃ and 25 ℃ respectively. Ectotherms, according to the temperature - size rule grow larger at lower temperatures (Angilleta and Dunham, 2003). This assertion was supported by Sibly and Atkinson (1994) pointing out that decreasing temperatures result in slower development rate, extended development time and larger adult size. On the other hand, lower temperatures can cause insects to stop feeding prematurely, a phenomenon that leads to smaller progeny sizes. Longevity, fecundity, sex allocation, morphology and behavior of parasitoids are all influenced by low temperature exposures (Hance *et al.,* 2007)

Insects are stressed when subjected to temperatures beyond their optimal and this induces behavioral and physiological changes that affect fitness traits (Angellita *et al.,* 2002; Chown and Terblanche, 2006). Extremely high temperatures beyond optimal is lethal to insects and the survivors of these temperatures compensate for it in their life history traits. Krebs and Loeschcke (1994) pointed out that, this may result in physiological change expressed as decrease in reproductive output, lifespan, changes in mating behavior and reduction or delayed growth especially when the immature stage is involved. Maynard (1995) and Hercus *et al. (*2003) observed that, hormesis (stimulating effect due to low levels of potentially harmful agents) as a result of repeated exposure of insects to mild heat stress may increase fitness cost by reducing fecundity via trade-off. In other studies, heat shocks in adults reduced proportion of egg hatching but not the viability of the hatched offspring (Silbermann and Tatar, 2000). Heat shock also induces changes in morphology (Andersen *et al*., 2005).

Temperature requirement of a parasitoid and its host affects the synchrony between the two. Differences in the preferences of thermal threshold and development temperature of both parasitoid and its host in future climates may uncouple the parasitoid-host interactions. For instance when parasitoid has a lower base temperature than its host in warm spring, the parasitoid could emerge earlier in the seasonal development of the host which will expose the host to relatively high numbers of adult parasitoid. The host population in that generation would be reduced effectively but the population of the future generation may explode since the parasitoid population in the year that follows would be crashed out as a result of limited resources (Hance *et al.,* 2007).

The extent at which temperature will affect the performance of insect herbivore as well as the relationship between them, their host plants and natural enemies in the future warming climate are not certain. However, many studies conducted revealed that, change in temperature definitely has an effect on insect development and as well will affect the trophic interaction between them, their host plants and natural enemies (Hance *et al.,* 2007). The role of temperature in the lives of poikilotherms is enormous making temperature perhaps the single most important environmental factor affecting insect distribution, development, behavior, survival and reproduction (Bale *et al.,* 2002). With some researchers believing that temperature effects on insects outweighs other environmental factors (Bale *et al.*, 2002), a study to find its impact on insect especially, natural enemies in the face of global climate change is inevitable.

Here I focused on the parasitoid, *Perilitus brevicollis*, (Hymenoptera: Braconidae), an important natural enemy to the detrimental Blue Willow Beetle, *Phratora vulgatissima* (Coleoptera: Chrysomelidae) in European short rotation coppices. Even though overlooked over the years, high parasitism rate (7-35%) has been observed in surveyed populations when field collections of *P. vulgatissima* was carried out in Sweden and Ireland in 2011 (Stenberg; Unpublished). With such high parasitism rate, the needed protection required by willow plants against the devastating *Phratora* beetle can probably be provided by the parasitoid hence affectionately called, the 'willow bodyguard'. Larval development of *P. brevicollis* is completed within the beetle. This gives the parasitoid the potential to be used as biological control agent on the beetle, *P. vulgatissima*, a major *Salix* pest. This beetle poses a serious threat to *Salix* for biomass production due to its frequency in reaching outbreak proportions in both natural stands and in *Salix* plantations (Björkman *et al.*, 2000a; Dalin *et al.*, 2009). However, the parasitoid's ability to be reared in captivity with temperature playing a vital role as well as its survival in the field will be crucial to its success as a biological control agent. This study assessed the impact of different constant temperatures on the performance of *P. brevicollis*. Development times and development rate of the progeny, adult parasitoid size, survival rate as well as host's feeding ability were measured. Parasitism rate among beetles from two populations (Sweden and Ireland) was also compared as well as adult parasitoid sizes. I hypothesized that:

1. Development rate of the parasitoid will be directly related to temperature rise
2. Adult parasitoid body size would be larger in lower temperatures
3. Parasitism would reduce the herbivore's feeding capacity more than low temperatures

Materials and Methods

Study Organisms

The host: *Phratora vulgatissima L.* (Coleoptera: Chrysomelidae)

Phratora vulgatissima is a common herbivore pest on *Salix* plants in Europe with a broad distribution in both Europe and Asia (Stenberg *et al*., 2010). It is found on several willow species (Maisner, 1974). In northern Europe, it is considered a major economic pest in willow plantations grown for biomass production (Kendall *et al*, 1996b; Björkman *et al*, 2003). *P. vulgatissima* is univoltine in Sweden and overwinters as adult. After their emergence from winter hibernation, adults appear and feed on host plants in early May (Dalin, 2004). Egg laying starts late in May until June with eggs deposited at the underside of the leaves in clusters of 10-50. Approximately, ≈ 7days days are required for eggs to hatch (Stenberg *et al*., 2010). Larvae pupate in the soil after going through three instars. Next generation of adult beetles emerged in August and with short period of feeding, finds overwintering sites under bark of trees, cracks of wood materials and in the ground vegetation.

Orthotylus marginalis Reuter (Miridae), *Closterotomus fulvomaculatus* De Geer (Miridae) and *Anthocoris nemorum* Linnaeus (Anthocoridae) are known common predators of *P. vulgatissima* (Björkman *et al.*, 2003). Other natural enemies like birds, spiders, pentatoid bugs may be abundant and important in other willow systems (Björkman *et al.*, 2003). Also *P. brevicollis* in recent times has been identified as a potential important natural enemy to the beetle.

The parasitoid: *Perilitus brevicollis* Haliday, 1835 (Hymenoptera: Braconidae)

We have limited knowledge about the biology of *P. brevicollis*. It is a solitary endoparasitoid that parasitizes *P. vulgatissima* and *Phratora vitellinae* (Coleoptera: Chrysomelidae). Oviposition occurs when adult female insert its ovipositor into the abdomen, behind the prothorax or around the scutellum of the host for some few seconds. It exhibits thelytokous parthenogenesis; females not necessarily have to mate in order to produce fertile offspring. High parasitism rate (7-35%) has been observed in surveyed populations when field collections of *P. vulgatissima* was carried out in Sweden and Ireland in 2011 (Stenberg; Unpublished). No other parasitoid has been found to parasitize adult *P. vulgatissima* in Sweden with the exception of one unidentified dipteran that parasitizes less than 1% of adult beetles (Stenberg; Unpublished). *P. brevicollis* over the years has been overlooked; however considering its high parasitism rate on adult *P. vulgatissima* as well as it's been a single important parasitoid species on *Phratora* in Sweden arguably makes it an important natural enemy to study.

Preparing the stock culture

Beetles used in this study were collected in the field from their overwintering sites on two occasions (11th January 2011 to 18th January 2011) from the bark of trees adjacent to a willow field in Kroksta,

North of Uppsala, Sweden (N 59° 55.925', E 17° 27.888'). Beetles were also collected from a population near Maynooth in Ireland (N 53°38', E -06°59'). Single populations from these two countries were used due to my inability in locating other beetle collection sites within the two countries. These beetles were shipped overnight to the Department of Ecology, Swedish University of Agricultural Sciences in Uppsala where both *P. vulgatissima* populations were kept in individual cages inside a greenhouse. The beetles were introduced on *Salix viminalis*, cultivar 78183 (Ager *et. al.*, 1986) grown in the green house. Eggs laid by the Swedish beetles were collected and incubated in transparent plastic containers (18cm X 18cm X 11cm). The eggs were reared to adulthood in the greenhouse and the purpose was to use the adults in the temperature experiment described below. The containers were lined with filter paper and pieces of water soaked oasis were kept in the container to provide moisture. Potted soil was placed at the bottom of the containers at the time when the third instar larvae were observed. This provided pupating substrate for the larvae. Fresh *S. viminalis* leaves were provided on every second day to ensure that larvae had fresh food during development. The feeding continued until adult beetles emerged. The matured adult beetles (1st generation population) were kept in the greenhouse at an average temperature of 23 °C and fed with fresh leaves until they were used in the temperature experiment. In order to obtain parasitoids for the temperature experiment, overwintering beetles obtain from the same location as the ones used in the 1st generation population beetles described above were introduced on *S. viminalis* plants raised in the greenhouse. *P. brevicollis* cocoons developed from these beetles were collected and placed in transparent plastic vial (30ml) with perforated lids and kept at the same temperature as the beetles until matured adult parasitoids developed. Adult parasitoids after their emergence, were fed with sugar solution and kept in the climate chamber (Thermo Kyl AB, Helsingborg, Sweden, model 3026/0) at 10°C until they were used in the experiment.

Experiments

The effect of temperature on parasitoid performance and host feeding capacity

The aim of this experiment was to assess how temperature impacts the development rate, body size, survivorship and host feeding capacity of *P. brevicollis* at four constant temperatures (10°C, 15°C, 20°C and 25°C). To do this, laboratory reared beetles were selected at random and parasitized (adult parasitoid powerfully thrusting the ovipositor into the host beetle). Each beetle was parasitized with a single parasitoid at a time. Each parasitoid was used to parasitize four beetles to reflect the four temperature levels used. In total, 120 beetles were used with 30 replicates per temperature level. Parasitized beetles were then introduced to the climate chambers (Thermo Kyl AB, Helsingborg, Sweden, model 3026/0) set at constant temperatures of 10°C, 15°C, 20°C and 25°C with 40 - 80 % RH under photoperiod, 19 : 5 L : D. Temperature log (SL54TH) was used to track the temperature in the chamber for the entire duration of the experiment. In two different occasions, the temperature in the chambers was altered due to technical faults. However, this lasted only for a few minutes.

For the entire duration of the experiment, parasitized beetles were provided with fresh *S. viminalis* leaves on a daily basis. The set up was observed everyday with development times (days) of the parasitoids from oviposition to larva (larval development) as well as from oviposition to adult (total development), recorded. Development rate as a reciprocal of the development time (1/days) was calculated from the development time. Hind tibia length of the adult parasitoid was measured with image analySIS Processing® software under a binocular (Olympus), as tibia length is a good indicator of body size and usually correlated with fitness trait (Godfray, 1994; Le Lann *et al.*, 2010).

In order to assess how temperature and parasitism affect the feeding capacity of the host, leaf area consumed (mm^2) by parasitized beetles were measured with a millimeter paper. The defoliated areas on the leaves were traced on the millimeter paper and the exposed squares (1mm x 1mm) were counted by the eye. A daily record of this from the day of oviposition until termination of the experiment was kept. Parasitoid survivorship (successful development of parasitoids from oviposition to adulthood) was calculated as the percentage of parasitoid adults emerged from pupae developed from adult parasitized beetles. The thermal constant (i.e. the minimum temperature) and the cumulative temperature (day-degrees) required for parasitoid larval development were calculated using a linear regression of developmental rate ($days^{-1}$ to pupation) plotted against temperature. The thermal constant was estimated by extrapolating the linear regression to the x-axis. The cumulative temperature requirement was calculated by multiplying the number of days to pupation with temperature minus the thermal constant. The mean from larvae surviving until pupation was used (n = 47).

Non parametric Kruskal-Wallis test was used to analyze the impact of temperature on development rate. A generalized linear model (GLM) with Poisson error structure was used to analyze the effect of temperature on body sizes of the developed parasitoids. An ANCOVA was used to analyze the impact of temperature and parasitism on host feeding capacity. Four-sample test for equality of proportion without continuity correction was used to test the difference in survivorship in the temperature levels used.

Performance of parasitoids from two different populations

The purpose of this part of the study was to compare rate of parasitism among beetles from one Swedish and one Irish population as well as identifying other parasitoid species from the Irish population. 402 adult field collected beetles from Ireland and 213 from Sweden were used. The beetles, collected from their overwintering stage were reared on potted *S. viminalis* plants grown in the greenhouse as described above between 25th February, 2011 and 8th April, 2011. The beetles were inoculated on the plants and kept in nylon net cage (92 cm x 47 cm x 47cm) to prevent beetles from one population to join the other. The number of *P. brevicollis* cocoons developed from these beetles as well as adults was recorded. Rate of parasitism was then calculated as the percentage of adult parasitoids developing from the total number of beetles used. Body sizes of both beetles and parasitoids from the two populations were measured in the same manner as was done in the temperature experiment described above.

A generalized linear model (GLM) with binomial error structure was used to analyze parasitism among Swedish and Irish populations. Depending on degrees of freedom and deviance (G^2), the p value was calculated on Chi-Square. The data on parasitoid size from the two populations did not conform to the

parametric assumptions for equal variance and normal distribution. As a result the data was analyzed with Mann–Whitney test. In comparing the sizes of beetles from the two populations, two sample T-test was used. With this data set, the parametric assumptions were fulfilled. The R software version 2.12.2 was used in analyzing the data in the two experiments described above.

Results

The effect of temperature on parasitoid performance and host feeding capacity

Development rate of parasitoids

Development rate increased with increasing temperature. Mean development rate was faster at 25℃ than with 10℃, the slowest (Fig. 1). There was a significant relationship between temperature and development rate ($p < 0.001$). Lower thermal threshold for development was calculated to be 6.95℃

with an average of 233±7 days required for development. Mean development time at each temperature level were: 62 ± 0.56; 29 ± 0.26; 17 ± 0.79; 12 ± 0.33 for 10℃, 15℃, 20℃ and 25℃ respectively.

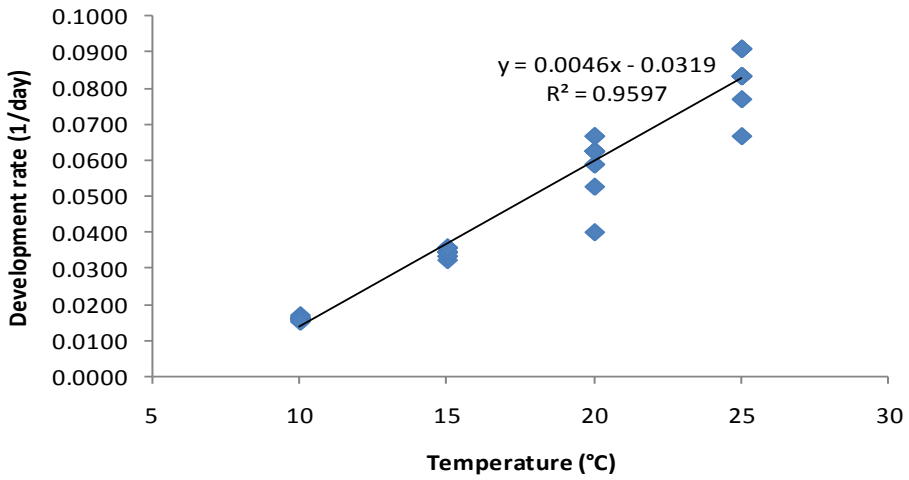


Fig. 1. Relationship between development temperature and rate of larval development of parasitoid *P. brevicollis* reared at four different temperatures (10℃, 15℃, 20℃ and 25℃).

Temperature effect on adult parasitoid size

Adult parasitoid size (hind tibia length) was found to be significantly affected by development temperature R^2=65.6, $p < 0.001$). This shown an inverse relationship as body size increased with a decreasing temperature (Fig. 2)

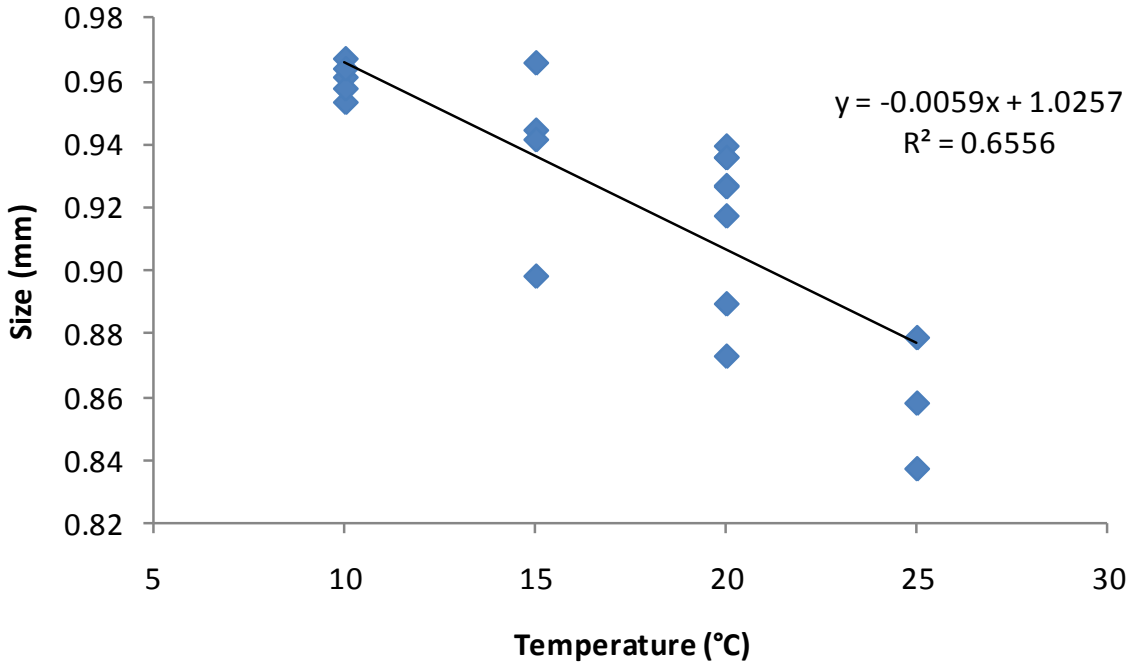
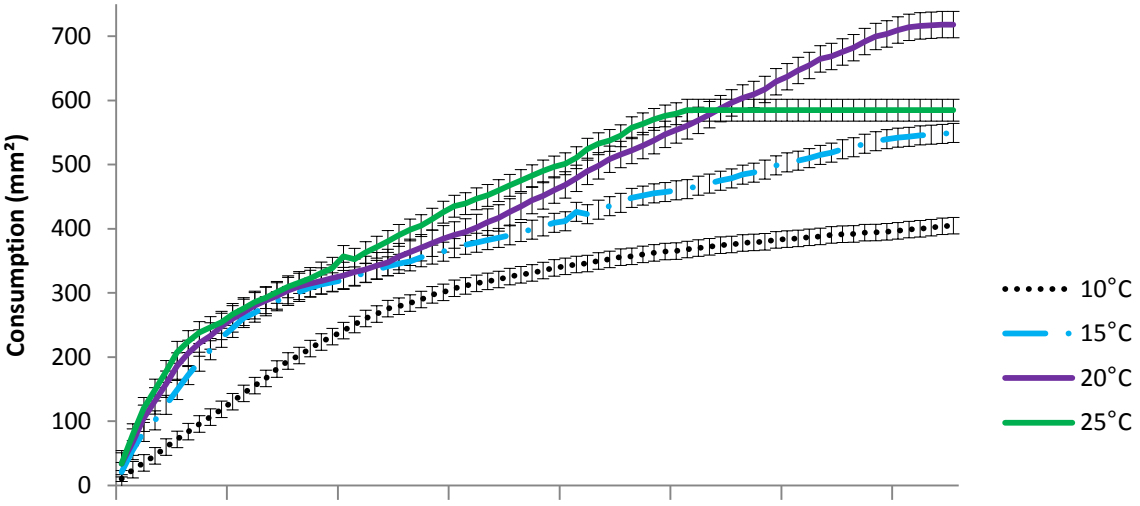


Fig.2. Relationship between adult parasitoid size and the temperature at which they were developed. *n*=5, *n*=5, *n*=8 and *n*=3 for 10℃, 15℃, 20℃ and 25℃ respectively.

Temperature and parasitism on host feeding

Temperature alone did not have a significant impact on the host's feeding capacity. However, parasitism significantly affected host's feeding capacity. Unsuccessfully parasitized hosts (beetles that do not have parasitoid emerging from them) fed more, compared to successfully parasitized hosts (beetles with parasitoids emerging from them). Temperature and parasitism together, significantly impacted on the hosts' feeding ability such that, at 10 ℃, the extent of feeding by both successfully parasitized (fig. 3b) and unsuccessfully parasitizes (fig. 3a) hosts did not differ much. However, at higher temperature levels a vast difference in feeding among successfully parasitized and unsuccessfully parasitizes hosts were observed.

a



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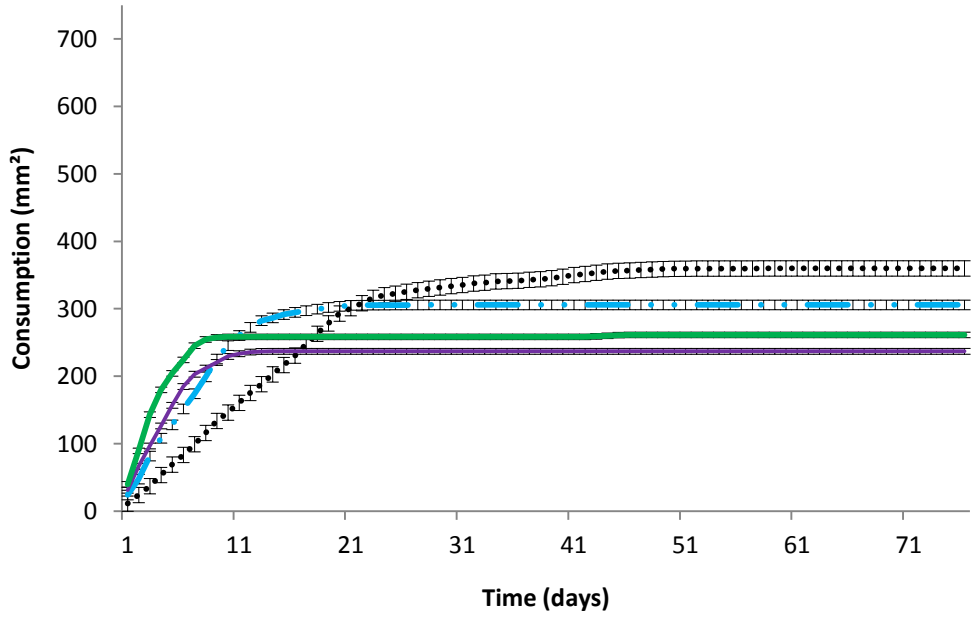


Fig. 3. Mean cumulative consumption (leaf area defoliated) of (a) unsuccessfully parasitized beetles and (b) successfully parasitized beetles in the four developmental temperatures. Leveling off of the graph indicates no further feeding due to death of the host either as a result of larval emergence or other unknown factors. Error bars indicate SE.

Table 1. Results of ANCOVA estimating the effects of feeding and parasitism on feeding capacity of leaf beetle *Phratora vulgatissima.*

Source of variation	*df*	*MS*	*F*	*p*
Parasitism (P)	1	1944053	17.8458	< 0.0001
Temperature (T)	1	216448	1.9869	0.16129
P*T	1	459187	4.2152	0.04
Error	118	12854469	108936	

Survival of the parasitoids under different temperatures

Temperature significantly impacted on the survival of the parasitoid from oviposition to adulthood. Relatively equal number of larvae, *n*=11, *n* = 11, *n*=12 and *n*=12 developed from the four temperature levels, 10℃, 15℃, 20℃ and 25℃ respectively. However, a significant difference in proportion (0/11; 5/11; 8/12; 3/12 for 10℃, 15℃, 20℃ and 25℃ respectively) of larvae survived to adulthood (X^2 = 12.30, *df* =3, *p* = 0.01). No adult parasitoid emerged from the 10℃ within the observational period (115 days). Slitting open of the parasitoid cocoons at the 10^0C revealed some fully developed adults that were alive. The cocoons at 10℃ appeared to have live parasitoid inside them for relatively longer period before they were slit opened. Even though some of the parasitoid reared at 10℃ were alive when the cocoons were opened, some were also dead with those alive looking very weak, making me believed that they might not have emerged alive should the cocoons have not been opened.

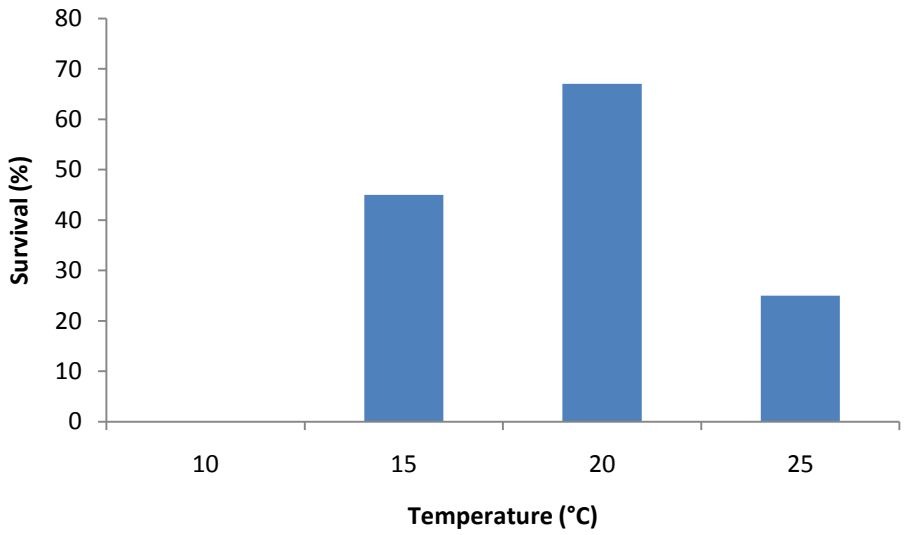


Fig. 4. Percentage survival of the parasitoid species under different temperatures.

Performance of parasitoids from two different populations

Rate of parasitism among Swedish and Irish populations

In comparing parasitism rate among the two populations, it was revealed that parasitism among the Swedish population was significantly different from the Irish population. (df = 1, G^2= 38.077 and p < 0.001). Higher parasitism rate was recorded: 27% in the Swedish and 8% in the Irish population respectively. The study also aimed at identifying different parasitoid species from the Irish populations. However, no other parasitoid species was found.

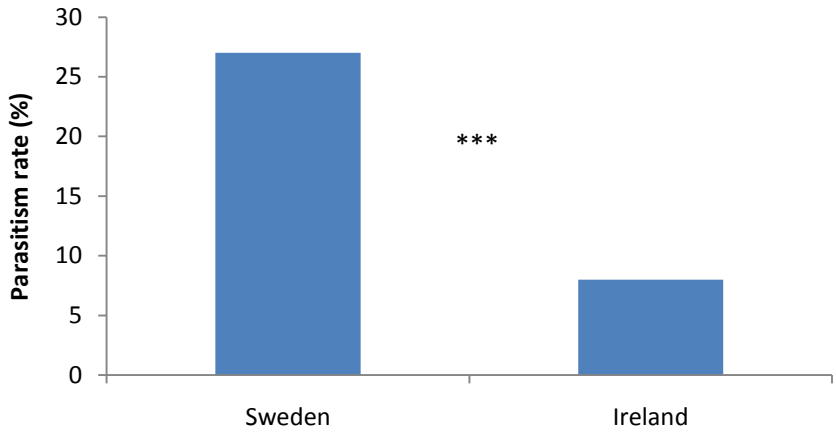
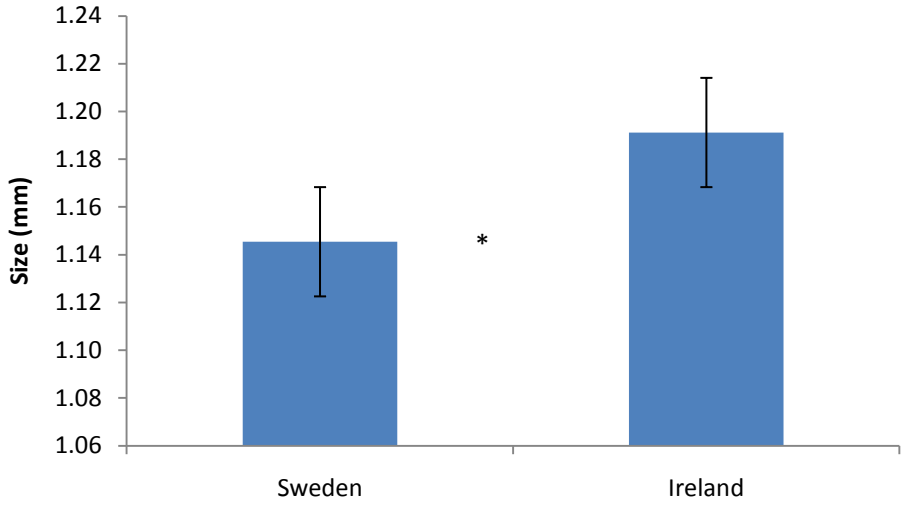


Fig. 5. Percentage parasitism among beetles from Swedish and Irish populations. *** indicate significant difference p<0.001. (n = 213 for Swedish population and n = 402 for Irish population)

Beetle and parasitoid size from different populations

Both the size of beetles as well as their respective parasitoid sizes shown significant difference among the two populations. Beetles from Ireland were significantly larger than beetles from the Swedish populations (df = 76, p =0.01). However, parasitoids from Ireland were smaller than those from the Swedish population (U=742, p = 0.03).

a



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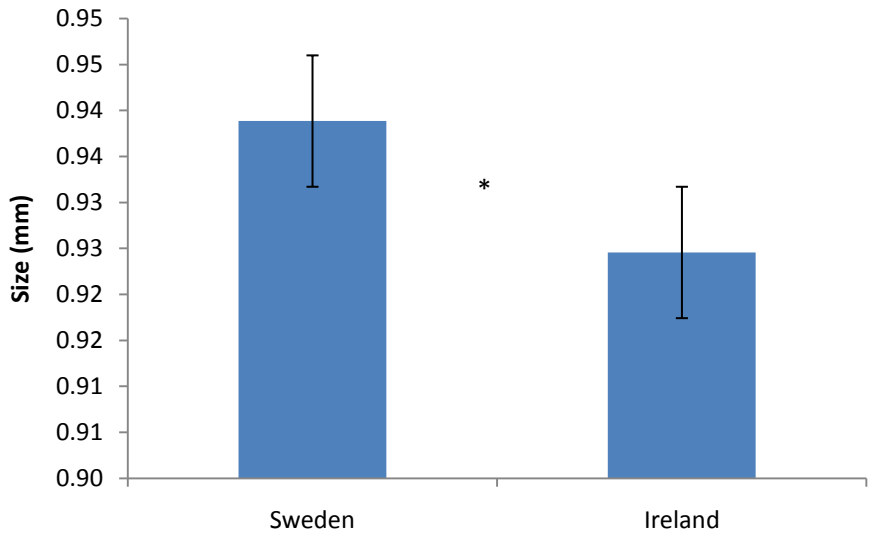

Fig. 6. Bar graphs showing (a) beetle and (b) parasitoid sizes from Swedish and Irish populations. *(n* = 40 and 38 for Swedish and Irish beetles respectively; *n* = 30 and 13 for Swedish and Irish parasitoids respectively) * indicate significant differences ρ= 0.05. Error bars indicate SE.

Discussion

Effects of temperature on parasitoid performance

Temperature plays a very important role among insects as it impacts their morphological and physiological processes. A study on how insects will fare under different temperature levels would be paramount in deciding which temperatures would be ideal for rearing the parasitoid for biological control purposes. This experiment aims at assessing how rearing temperatures affect the performance of *P. brevicollis.* The study also aimed at how temperature will impact the feeding capacity of the parasitoid host, *P. vulgatissima.* The study did not only revealed that rearing temperatures affect development rate and time but also affect the final adult size attained by the parasitoid and the survival rate of the parasitoid. When feeding capacity of the host was observed, it was realized that parasitism and temperature have additive effect on reducing host feeding capacity, phenomena that may make *P. brevicollis* an effective biological control agent in future warming climates.

Temperature on development rate and time

Among insects, development rate increases almost linearly with temperature, however, it may decrease at extreme temperatures due to physiological stress (Gilbert and Raworth, 1996; James and Warren, 1991; Charnov and Gillooly, 2003).

Under the temperature range studied, my results also show linearity between temperature and developmental rate in the parasitoid, *P. brevicollis*. This pattern suggests that, increase in temperature would benefit the parasitoid since this will mean a reduced development and generation time. One possibility is that the parasitoid could have more generations per season and hence increase population growth. However, the increased development rate at high temperatures can probably have negative effects as well if the parasitoid body size is affected by temperature (see below). Longer development time or slow development rate favors fecundity via larger adult size since larger adult sizes correlates with amount of eggs produced by female parasitoid as well as egg load (Godfray, 1994). The parasitoid could then select for genotypes that will promote slow development at initial stages to ensure optimal adult size (Gilbert and Raworth, 1996) to enhance fecundity and at the same time develop faster at later stages so as to reduce its generation time to promote faster population growth. Whether this will benefit the parasitoid more than its host in future climates cannot be ascertained since enough studies on how both the parasitoid and its host will simultaneously react to climate change is lacking.

The thermal threshold as well as accumulated degree days for this study was calculated to be 6.59°C and 233 ± 7 days respectively for the parasitoid. Comparing this to the thermal threshold and accumulated degree days (5.5 and 222 days respectively) for the host as calculated in Dalin (2011) revealed a higher thermal threshold and constant for the parasitoid than the host. This is in agreement with what is expected in the field since parasitoids should not emerge from their winter dormancy until their first prey has appeared hence having a higher thermal threshold than the host (Gilbert and Raworth, 1996). The difference in the thermal constant exhibited by the parasitoid and its host makes *P. brevicollis* a potential biological control agent for *P. vulgatissima*.

Temperature on parasitoid size

The temperature – size rule predicts thermal reaction norm for final body size to be negative. Ectotherms developing at higher temperatures will be relatively smaller (Atkinsson, 1994, Angelitha and Dunham, 2003). Out of 109 studies reviewed by Atkinson on diverse taxa including arthropods (80 studies), 83% exhibited negative reaction norms (Kingsolver and Huey, 2008, Atkinson, 1994). My study is in conformity with this since adult body size of the parasitoid decreased with an increasing temperature. This pattern can be explained by an organism's thermal sensitivity to growth and development rates. Since higher temperatures result in faster growth and development rates, if an organism's thermal sensitivity to development rate tends to be greater than growth rate, then small body size would be expected. In future warmer climates, it is not clear how the parasitoid will be affected in its thermal sensitivity. Based on this study, it is expected that parasitoids will have smaller body sizes since development rate turned to be faster at higher temperatures with a corresponding small adult sizes. Despite the fact that smaller body sizes negatively affect mating success (Kingsolver and Huey, 2008), I do not think this will greatly affect the population growth of *P. brevicollis* since the females undergo thelytokous parthenogenesis.

Temperature on survivorship

As was shown in the study, parasitoid survival from oviposition to adulthood was significantly affected by rearing temperature. Survival appeared to be higher at 20°C followed by 15°C with 10 and 25°C performing poorly. This is in accordance with other studies where very low survival rate was recorded at 10°C with 20°C as the highest peak beyond which a decline in survival was recorded (Tun-Lin *et at.*, 2001). Rueda *et al.* (1990) in their study also recorded similar pattern. Even though the above mentioned studies were performed on different insect orders, the common thing here is that, rearing temperatures affect survivorship and there seem to be an optimal thermal limit within which survivorship is successful. Thus to consider *P. brevicollis* in biological control, the identification of this optimal rearing temperature for the parasitoid is inevitable. Based on the findings from this study, 20°C seems to be ideal for *P. brevicollis* since at this temperature performance (survival and growth rate) was higher. Haven said this; it could also be laudable for other studies to be conducted in the field as well as the laboratory to find out how other biotic and abiotic factors could impact on the parasitoid's survival.

Temperature and parasitism on host feeding

From the study, parasitism significantly affected the feeding capacity of the host at all temperatures except at lower temperature (10 °C) where feeding capacity seemed not to have changed much between both successfully parasitized and unsuccessfully parasitized hosts (Fig. 3a & b). Temperature alone on the other hand did not significantly affect the host's feeding capacity even though feeding increased with increasing temperature among unsuccessfully parasitized host (Table 1 and Fig. 3a & b). However, the combine effects of temperature and parasitism had significant effect in reducing the host's feeding capacity suggesting that, interactive effect of temperature and parasitism is effective in reducing host's feeding at higher temperatures than in the lower temperatures. Increasing temperatures favor parasitism as was observed by Burnett (1951) and since parasitism was effective in reducing host feeding at higher temperatures in this study, should the global climate become warmer in future, *P. brevicollis* would be effective in controlling *Phratora* damage in *Salix* plantations.

Performance of parasitoids from two different populations

The purpose of this study was to identify similarities and differences among the two populations with regard to parasitoid diversity, body size and parasitism rate. This comparison was done not for the purpose of making factual predictions but to provide baseline information upon which further detailed study can be conducted. Even though no other parasitoid was found among the two populations studied, the size of the parasitoid as well as their hosts were different; so as the rate of parasitism.

Among insects, the converse Bergmann's rule where insect body size decrease with increasing latitude (altitude) has been observed in some studies (Mousseau 1997; Nylin & Svärd 1991). Explanation to this trend has been attributed to interaction between seasonal length and physiological time for development rather than thermal gradient (Mousseau 1997). As was pointed out by Mousseau (1997), higher latitudes have relatively shorter growing season than lower latitudes and as such, development time among univoltine species from the higher latitudes are much constrained than in the lower latitudes. Giving the fact that development time is positively related to insect body size if it is constrained, then smaller sizes would be expected. On the other hand, other studies counter this rule suggesting that ectotherms follow Bergmann's rule (Chown and Gaston, 1999 & Blanckenhorn and Demont, 2004). Even though beetles used in my study were from two populations with varied latitudes, the fact that single populations from each location was considered makes it dicey to relate the results with findings from the aforementioned studies let alone to make any general predictions from it. Likewise the hosts, the parasitoids also had differences in their sizes among the populations studied with the population from Sweden bigger than the population from Ireland. Again the result could be treated in the same way as the hosts'. However, the results obtained could serve as a basis for further studies where several populations within and across geographic areas with different latitudes could be considered.

Conclusion

This study revealed that higher rearing temperatures result in faster development rate and time of the parasitoid whereas lower temperatures work to the contrary. In future climate where temperatures are expected to rise, this may contribute to the reduction of the parasitoids generation time and hence increase the buildup of the parasitoid population in short time, something that makes the parasitoid effective in controlling its host's population. Size and survival of the parasitoid were also affected by rearing temperature such that, the adult parasitoids reared at higher temperatures were smaller in size than those reared at lower temperatures. Parasitoid survival was also higher at higher temperatures, even though the highest temperature (25 ℃) was not favorable. 20 ℃ could be suggested from this study as an ideal temperature for rearing the parasitoid since at this temperature, higher survival as well as relatively larger parasitoids sizes were achieved. This study also predicts that, future warmer climates may count to the favor of the parasitoid in controlling the beetle population since the combined effects of temperature and parasitism reduced host feeding especially at the higher temperatures. The identification of the same parasitoid species from both Swedish and Irish population is an indication that, the use of *P. brevicollis* in biological control would not be limited to Sweden. However, it would be laudable to conduct further studies in the field in geographic areas where *Salix* is planted to ascertain the abundance of the parasitoid as well as their performance in those areas. Having said this, it is also worth noting that, the actual cause in difference in rate of parasitism and parasitoid sizes across latitudinal gradients need further studies.

In conclusion, I would say, this study has provided enough background information that can be used in predicting the performance of the parasitoid as well as its success in mass rearing for biological control programs.

Acknowledgement

I would like to express my appreciation to my supervisors Johan Stenberg and Peter Dalin for their guidance right from the inception of the study to the end. Valuable comments on a previous version of the thesis were also provided by my examiner, Göran Nordlander. Faisal Kabir also needs a mention for his assistance in collecting the field beetles during the cold Swedish winter. The study was funded by the Swedish research council, Formas.

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